

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

The Change of Oxidation-Reduction Potentials of Water-logged Soils. III.

Humus- alkali- and fish farm soils.

(pp. 529~536)

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In the previous paper (J. Agr. Chem. Soc. Japan, (1936), **12**, 62; **12**, 1141), the authors reported the result of general investigation on the change of oxidation-reduction potentials of different soils which were placed under water-logged condition for 100 days at 30°C, and the result of a further investigation on factors which affect on the change, using a lateritic- and a sandstone-shale soil in Taiwan. The present paper deals with the change of oxidation-reduction potentials of characteristic soils under the same condition. These soils are a humus-, an alkali and a fish farm soil, taken from different districts of Taiwan.

The following tests have been carried out by this investigation.

- (1) Change of oxidation-reduction potentials, reaction and FeO content of the soils which were placed under water-logged condition after varying their pH values.
- (2) Change of oxidation-reduction potentials and reaction of the soils which were in water-logged condition after addition of $HgCl_2$ solution, in order to prevent microbiological activities.
- (3) Change of oxidation-reduction potentials and reaction of the soils which were in water-logged condition after addition of mannit to 20 per cent of the soils.
- (4) Change of oxidation-reduction potentials and reaction of mixtures

of water extracts of the soils and mannit solution. The mixtures were made by mixing of mannit in the water extracts, in the same proportion to that applied to the soils. These mixtures were kept for about 100 days at 30°C under the anaerobic condition.

(5) Change of oxidation-reduction potentials and reaction of the same mixtures as described above, but containing $HgCl_2$ to extent of 1000 p.p.m.

The result of this investigation is to be summarized as it follows :

(1) Oxidation-reduction potentials of the soils which were varied their pH values, respectively, from alkaline to acid or from acid to alkaline, markedly declined within the first week, under water-logged condition. The depression, thereafter, slowly and insignificantly continued. Ferrous oxide determined in the soils gradually increased, in accordance with advancement of the water-logging period. It was noticed that oxidation-reduction potentials of the soils which were artificially varied their reactions, similarly changed themselves to those of the original soils under water-logged condition.

(2) Oxidation-reduction potentials of the soils which were added $HgCl_2$ solution and were placed under water logged condition for about 100 days, depressed themselves to some extent. Nature of the potential depression was, however, a little different from that of the soils without addition of $HgCl_2$. It was almost similar to the potential change of pure sesquioxide of iron, showing not significant depression within the first week. It should be considered, therefore, that the potential change of the soils which were added $HgCl_2$ solution depends upon reduction of mineral constituents of soils as sesquioxide of iron under water-logging condition.

(3) Mannit was added, on the other hand, to the soils as a nutrient of micro-organisms, in order to exert their activities. Oxidation-reduction potentials of such soils markedly declined under water-logged condition, as they displayed in the original soils, noticeably depressing themselves in the earlier period of water logging.

(4) The potentials of mixtures of the water extracts of soils and mannit solution similarly depressed themselves at the beginning of the period.

(5) When the water extracts were mixed with mannit and $HgCl_2$, the potential change was insignificant.

From these experimental evidences, in short, it should be said that the depression of oxidation-reduction potentials of soils under water logged condition is principally brought about by the activity of micro-organisms at earlier period of water logging, while it mostly depends upon reduction of such mineral soil constituents as iron salts, at the later period. It is, occasionally, noticed that the potentials of water-logged soils again rise up to a small extent at about 5 weeks after submerging the soils. This is to be attributed to a certain formation of organic acids by the micro-biological decomposition

of organic matter in the system, although there is equivalent compensation between the newly formed acids and the reduced mineral soil constituents.

On the Refractive Index of the Hen's Egg-Albumin. (Part 1.)

(pp. 537~545)

By Kinsuke KONDO and Hiromu IWAMAE.

(Laboratory of Nutritional Chemistry, Kyoto Imp. University; Received April 24, 1937.)

1. The writers showed that the refractivity and the specific viscosity per a certain amount of the hen's egg-albumin in the solution varied linearly with the concentration of the albumin. These phenomena were the same as those observed in the solutions of the rice-gluteline, the casein and the egg-albumin of the loggerhead turtle which were already reported by the writers. Then, the refractivity of the protein solution does not vary proportionally with the concentration of the protein. This is opposed to the classical theory of Robertson. The reasons and causes were explained.

2. The refractivity, the viscosity and the specific gravity per a certain amount of the albumin in the ammonium sulfate solution vary with the concentration of the ammonium sulfate. It is caused by the variation of the ionization, the electric charge, the hydration, the swelling and the volume contraction of the protein molecule, and these causes are the same as occurred in the case of (I).

(October 1, 1936)

On the Refractive Index of the Hen's Egg-Albumin. (Part 2.)

(pp. 546~553)

By Kinsuke KONDO and Hiromu IWAMAE.

(Laboratory of Nutritional Chemistry, Kyoto Imp. University; Received April 24, 1937.)

I. The writers observed the changes of the refractivity and the specific viscosity of the hen's egg-albumin water-solution when the paH-value of the solution were varied by the addition of the dilute sulfuric acid or ammonia, and results are as follows.

(a) The refractivity of the albumin water-solution varies with the change of paH-value, and the refractivity increases to maximum at an iso-electric point of the albumin, and varies remarkably on an iso-electric zone.

(b) The specific viscosity of the albumin water-solution varies reciprocally with the refractivity.

(c) Such a variation was caused in the changes of the ionization, the electric charge, the swelling and the hydration of the albumin molecule, and

such a change was caused in the change of the paH-value of the solution.

II. The refractivity, the specific gravity and the specific viscosity of the albumin in the ammonium sulfate solution varied with the change of the paH-value of the solution. These variations occurred remarkably on the iso-electric zone. The refractivity and the specific gravity of the albumin in the ammonium sulfate solution increased to maximum at the iso-electric point and decreased suddenly on the acidic side of the iso-electric point and then increased again, if the solution became more acidic. But, on the alkaline side of the iso-electric point, the refractivity and the specific gravity of the albumin decreased twice suddenly and then increased again with the paH-values. The specific viscosity of the albumin varied reciprocally with the refractivity. The causes of such changes as described above were considered as follows.

(a) At the iso-electric point, the albumin molecules take form of zwitter ion and fully ionize, hydrate and swell through the dissociation in components, the ionization and the hydration of the albumin are controlled by the ammonium sulfate. This is the cause for the increase of the refractivity and the specific gravity of the albumin.

(b) If the solution of the ammonium sulfate solution remove from the iso-electric point, the refractivity and the specific gravity of the albumin decrease suddenly, because the ionization is controlled. On the contrary, if the reaction of the solution remove far from the iso-electric point, the refractivity and the specific gravity increase, because the ionization is accelerated and the electric charge increases. But, if the paH-value be near 2 or 8, the irreversible dissociation in components and the racemization of the component-proteins may take part in the variation of the refractivity and the specific gravity of the albumin.

(October 10, 1936)

On the Isoionic Reaction of the Hen's Egg-Albumin.

(pp. 554~557)

By Kinsuke KONDO and Hiromu IWAMAE.

(Laboratory of Nutritional Chemistry, Kyoto Imp. University; Received April 24, 1937.)

1. The writers determined the changes of the paH-values of the conc. ammonium sulfate solutions when the solutions were added with dil. sulfuric acid or dil. ammonia. Then, the paH-values of such solutions as above mentioned, but containing about 2% of the albumin, were determined.

2. Comparing the above results, the writers discovered that the albumin combined with sulfuric acid or ammonia, and the ammonium sulfate combined also with sulfuric acid or ammonia, though the latter combination was not so strong as the former.

3. The isoionic reaction of the albumin was dependent on the ammonium sulfate. The causes of this dependence were discussed.

4. If the influences of the salts and albumin itself are absolutely zero, the isoionic reaction of the hen's egg-albumin would be 4.89 ± 0.02 as the value of pH . (December 4, 1936)

Studies on a Mannitol-Bacteria.

(pp. 558~568)

By Bensiro NOMURA.

(The Agricultural Chemical Laboratory, Tokyo Imp. Univ.; Received May 4, 1937.)

In this work it was found that this mannitol-bacteria had different character morphologically and physiologically from the others, which were isolated up to date. So the writer proposed to name this microbe "Bacterium mannitpoem var. batatas."

Chemical Studies on Green Tea. I.

Comparison between Chemical constituents of Mecha and Bancha.

(pp. 569~578)

By Saichi MACHIDA.

(Chemical Laboratory, Tokyo Agricultural College; Received April 28, 1937.)

Chemical Studies on Japanese Coccidae. XVI.

On the Carbohydrates and Waxes of Sasakiaspis pentagonia Tar.

(pp. 579~585)

By M. Kawano and R. MARUYAMA.

(Laboratory of Osaka Factory of Sankyo Co. Ltd.; Received April 30, 1937.)

On the Koji-Amylase. (Part IX.)

The experimental Proof of the Presence of β -Amylase in Koji.

(pp. 586~594)

By Y. TOKUOKA.

(Received May 10, 1937.)

Series of experiments were carried out in the present paper, in order to test whether β -amylase could be found in Koji with which the presence of

β -amylase was not yet definitely been ascertained, although α -amylase and maltase were already proved to be existed.

(1) When Koji (Asp. oryzae grown on steamed rice) was extracted with 1% sodium chloride solution, adjusted pH value of the extract to 5.6 \sim 6.0, added alcohol up to 40% and a small amount (nearly 1% of dry matter) of residual substances of Koji being thoroughly extracted enzymes with the salt solution, α -amylase was almost quantitatively absorbed by the residual substances of Koji. The absorbed α -amylase was extracted with salt solution from the absorbent after being repeatedly washed with 40% alcohol, and then purified by precipitation with 65% alcohol.

The preparation was found to be free from maltase and 60 \sim 75% of amylase could be recovered from the extract, therefore the present method of preparation of α -amylase would be very convenient and more improved than the previous one [see Part IV. This Journal. 12, 1199, (1936)].

With the α -amylase preparation, the rate of decomposition of starch was at first found to be very rapid according to monomolecular reaction until degree of decomposition reached to 37% when iodine coloration was almost disappeared, and then gradual decomposition was taken place until reducing power, calculated as maltose, reached to 70% of the theoretical value.

During the first and rapid stage of decomposition, the velocity constant was, no doubt, found to be proportional to the amount of the preparation.

(2) After being ground Koji with the same amount of water, added alcohol up to 50%, and enzyme preparation was obtained from the filtrate of the ground mash by precipitation with 75% alcohol.

Although 68% of Koji-maltase was recovered in the enzyme preparation, any trace of α -amylase was not suggested to be existed in the preparation, since greater amount [nearly 50 times of the former (1) case] of absorbent was employed.

However, very remarkable decomposition of starch, was taken place with the enzyme preparation, although violet iodine coloration was still found to be remained even when decomposition attained to 65% (dextrinising power was calculated to be nearly 1/250 of that of the salt extract of Koji), and liquefying power was observed to be about 1/30 of the α -amylase preparation above mentioned.

These results of experiments show very probable evidence for the presence of β -amylase in the enzyme preparation.

(3) In order to ascertain whether β -amylase would again reveal maltase activity, series of experiments were carried out by absorption and elution method, since with the enzyme preparation above (2) mentioned, maltose and starch were decomposed at nearly the same rate as 3:5.

The residual substances of Koji was again found to be very useful

absorbent for maltase and β -amylase in the absence of sugars (glucose and maltose), so that the enzymes were absorbed by the residual substances of Koji (nearly 15% of dry matter) from the enzyme solution (2) at optimum pH = 4.3, after being added alcohol up to 50%, and the absorbent, thus obtained, were several times washed with 40% alcohol.

The aqueous elution of the absorbent revealed greater maltase activity and least amylase power as was calculated to be the ratio 5:1, while the ratio of the activity of the enzymes in the elution of 1% sodium chloride solution, after being washed the absorbent with water, was found to be 1:3.

From the natures of these enzyme elutions, Koji-maltase and Koji β -amylase would be concluded not to be identical but the two enzymes were different from each other.

(4) Even when α -amylase preparation (1) was added to the aqueous elution (3) as to reveal the same dextrinising power of the salt elution (3), saccharifying activity was still found to be much inferior (nearly 1/3) to the salt elution which contained least amount of maltase than the aqueous elution.

These results would propose another experimental proof of the presence of β -amylase, since activity of the salt elution could not be attributable to maltase and α -amylase, but to be principally due to β -amylase.

Chemical Studies on Silk Fibroin. (VI.)

The relative viscosity of Fibroin and its component solution (2)

(pp. 595~600)

By Hideo KANEKO and Yoshio NAKAZAWA.

(College of Sericulture and Silk Industry, Ueda; Received May 1, 1937.)

It is well known that silk fibroin dissolves rapidly in conc. H_2SO_4 solution and yields a viscous, slight yellowish solution which shows extremely high viscosity. This high relative viscosity of fibroin sol is mainly due to the special structure of fibroin micelles and in part due to the higher solvation. The micellar structure of fibroin is gradually disintegrated into the state of its components with rising the temperature, elapsing time and increasing the amount of water content in H_2SO_4 solution after dissolution. On the other hand, fibroin component sols exhibit a low viscosity under the same condition. This is attributed to the fact that fibroin components have no normal characteristic structure of fibroin by the destruction with HCl used as the dispersing agent for fibroin fibre and that they undergo into lyophptic state during their drying. So the fibroin components are reduced their

solubilities in conc. H_2SO_4 and also the degree of solvation as shown in the following table.

at 10 C. in 70% H_2SO_4 solution.

	Fibroin	Fibroin A	Fibroin B
Kc	0.421	0.117	0.117
φ	40.1	36.9	32.2
b	1.66	0.82	0.60

where Kc is mean viscosity-concentration constant, φ specific volume in c.c. and b degree of solvation.

Studies on Canavanin. (VIII.)

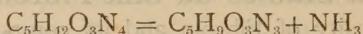
(pp. 601~612)

By Matsunosuke KITAGAWA and Jiro TSUKAMOTO.

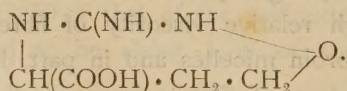
(Biochemical Laboratory, Kyushu Imperial University, Fukuoka, Japan;

Received May 8, 1937.)

Canavanin in the free state easily liberates one molecule of ammonia on heating in the aqueous alcoholic solution to convert into a intramolecular condensation product of the molecular formula, $C_5H_9O_3N_3$, which was named desamino canavanin after the mode of the formation. On the other hand, canavanin is stable on prolonged boiling with 10% HCl. The above change of canavanin is shown in the following equation :



This product indicates a distinct Sakaguchi's reaction for guanido fatty acid and not ninhydrin reaction, differing from canavanin. It decomposes copper acetate to form copper salt and gives ester with alcohol. On prolonged hydrolysis by acid, it ultimately converts into canalin at a loss of ammonia. From these facts, we assume for its constitutional formula :



This reaction of canavanin already takes place at a comparatively low temperature, namely, at 28°. That some preparation of canavanin sometimes indicates Sakaguchi's reaction, is due to the contamination of this compound, which is spontaneously produced from canavanin during the process of its isolation.

**On the Production of Vitamin C-like Reducing Substance
by Mouldfungi.**

(pp. 613~620)

By Juichiro FUKUMOTO and Hiroshi SHIMOMURA.

(Department of Food Chemistry in the Osaka Municipal Institute of Industrial
Research Received ; May 15, 1937.)

1. Authors studied on the production of the reducing substance, characteristic for the reduction of 2, 6-dichlorphenolindophenol and iodine by mouldfungi. Plenty production of the reducing substance were observed in the culture media of *Asp. cellulosa*, *Asp. fumigatus*, *Asp. niger*, *Asp. nidulans*, *Asp. melleus*, *Pen. glaucum* and *Pen. luteum*.

2. The suitable C-source for the production of the reducing substance by *Asp. niger* were fructose, sucrose, mannit and starch.

3. By feeding experiment with this reducing substance on guinea-pigs, authors proved that this reducing substance had no physiological effect like vitamin C.

4. Some chemical natures of this reducing substance were discussed.

Studies on Crystalline Urease. (I.)

(pp. 621~628)

By Matsunosuke KITAGAWA and Minoru FUJII.

(Biochemical Laboratory, Kyushu Imperial University, Fukuoka, Japan ;

Received May 20, 1937.)

It was known that the crystalline urease could not be obtained at all from Japanese Jack bean by Sumner's acetone method. Recently we knew that American Jack bean—a good material for the isolation of crystalline urease—was of the erect variety, while our Japanese Jack bean was of the twining variety, which was widely distributed in Japan.

Then the erect variety of Japanese Jack bean, which was cultivated rarely in Japan, was tried to be used for the isolation of the crystalline urease, so the octahedral crystals of urease could be obtained from the acetonite extract by Sumner's method.

Now the total urease content of Jack bean and its fraction to be extracted by 31.6% acetone were tested about three of the erect variety and one of twining variety. The results are shown in the following table, in which urease unit is expressed by N mg of ammonia produced by urease at 20° for 20 minutes.

	American erect variety.	Japanese erect variety.		Japanese twining variety.
		A	B	
(1) Name of variety of Jack bean.				
(2) Total urease units per g meal. (N mg)	965	953	904	776
(3) Urease units per c.c. acetonic extract. (N mg)	103	59	30	20
(4) Urease units extracted by acetone per g meal. (N mg)	576	331	168	112
(5) Ratio of extractive to total urease. (%)	60	35	19	14
(6) Sumner's units of the preparation of urease by his method.	77464	31812	15964	3520
(7) Existence of octahedral crystals.	+	+	—	—

From this table, it is known that three samples of erect variety contain almost same amount of urease, the amount of urease extracted by 31.6% acetone, however, is respectively different according to their origin.

According to our several experiments, it was ascertained that the crystalline urease could never be obtained from the 31.6% acetonic extract of Jack bean, unless urease units of the extract amounted to about 58. On the other hand, the crystalline urease was known to be more easily soluble in 31.6% aceton, or water, than urease separated in the amorphous form.

From these results, we assume that Jack bean contains two kinds of urease, that is, one is crystallizable and easily soluble in a dilute aceton, or water, and the other amorphous and less soluble in these solvents. The ratio of the quantity of two kinds of urease to each other in Jack bean is considered to be respectively different according to the origin of Jack bean.

Studies on "Funasushi." Pickles of Crucian in Boiled Rice.

(pp. 629~634)

By Kenji MATSUSHITA.

(Agr. Chemical Laboratory, Kyoto Imperial University, and Kurita School of Agriculture, Shiga, Japan; Received May 27, 1937.)

Part I.—Analysis of "Funasushi."

Noticeable diminution of protein and fatty substances was found, when crucian was pickled in salt and then washed by water.

When salted crucian was again pickled in boiled rice, production of sugar by Koji-amylase, lactic acid and alcoholic fermentations were observed to be taken place.